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☐ 1: Cell 1989 Jun 2;57(5):847-57

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Recovery of *Agrobacterium tumefaciens* T-DNA molecules from v plants early after transfer.

Bakkeren G, Koukolikova-Nicola Z, Grimsley N, Hohn B.

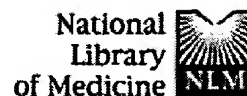
Friedrich Miescher-Institut, Basel, Switzerland.

A system for the analysis of independent T-DNA transfer events from *Agrobacterium tumefaciens* plants is described. The complete T-DNA except for the 25 bp border sequences replaced by one genome of a plant virus so that upon transfer to the plant, a viral replicon is produced by circularization. Rescue of virus from such infected plants: analysis of DNA sequences at or close to the ends of T-DNA molecules. A rather right border remnant of three nucleotides was found, whereas the sequences at the left end were more variable. A point deletion in the left 25 bp sequence resulted in less precise processing at the left end. In addition, many rescued T-DNA molecules contained small direct repeats between the joined T-DNA ends; linear T-DNA molecules are transported to the plant.

PMID: 2720788 [PubMed - indexed for MEDLINE]

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☐ 1: Plant Cell 1996 May;8(5):873-86

Related Article

Early transcription of Agrobacterium T-DNA genes in tobacco and

Narasimhulu SB, Deng XB, Sarria R, Gelvin SB.

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 4 USA.

We developed a sensitive procedure to investigate the kinetics of transcription of Agrobacterium tumefaciens transferred (T)-DNA-encoded beta-glucuronidase gene soon after infection of plant suspension culture cells. The procedure uses a transcriptase-polymerase chain reaction and enables detection of gusA transcripts 18 to 24 hr after cocultivation of the bacteria with either tobacco or maize cells. Expression of gusA transcripts depended absolutely on the intact virulence (vir) genes virB, virD1/virD2, and virD4 within the bacterium. Mutations in virC and virE resulted in highly attenuated expression of the gusA gene. A nonpolar transposon inserted into the C-terminal coding region of virD2 resulted in only slightly decreased production of gusA mRNA, although this insertion resulted in the loss of the nuclear localization signal and the important omega region from VirD2 protein and rendered the bacterium avirulent. However, expression of gusA transcripts in tobacco infected by this virus was more transient than in cells infected by a wild-type strain. Infection of tobacco with an Agrobacterium strain harboring a mutant virD2 allele from which the omega region had been deleted resulted in similar transient expression of gusA mRNA. These results indicate that the C-terminal nuclear localization signal of the VirD2 protein is not required for nuclear uptake of T-DNA and further suggest that the omega domain of VirD2 is required for efficient integration of T-DNA into the plant genome. The findings that the initial kinetics of gusA gene expression in maize cells are similar to those shown in tobacco cells but that the presence of gusA mRNA in maize is highly transient suggest that the block to maize transformation involves T-DNA integration and not T-DNA nuclear targeting.

PMID: 8672885 [PubMed - indexed for MEDLINE]

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